



## ROLE OF D-RIBOSE-L-CYSTEINE IN PREGNANT SPRAGUE-DAWLEY RATS ADMINISTERED NICOTINE

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### ABSTRACT

Nicotine a major constituent of cigarettes has been found to impact pregnancy in any trimester. It crosses the placenta barrier and has been found in the amniotic fluid and fetal blood. This study determined the role of D-Ribose-L-Cysteine (DRLC) a compound that enhances the production of intracellular glutathione on nicotine-induced toxicities during pregnancy. Twenty-eight pregnant Sprague-Dawley rats were used for this study. The rats were divided into four groups; control, nicotine, nicotine + DRLC and DRLC alone group. DRLC (200 mg/kg orally) and nicotine (0.5 mg/kg subcutaneously) was administered from day 4 to day 18 of pregnancy. The rats were sacrificed on day 19 of pregnancy and blood pressure was measured in the rats using the invasive method. Blood was collected to estimate liver enzymes and c-reactive protein (CRP) concentrations. The placenta was homogenized and used to estimate the oxidative stress indices. Nicotine reduced the fetal and placenta weights of the rats and increased blood pressure. Co-administration of nicotine + DRLC increased the fetal and placenta weights significantly and reduced the increased blood pressure of the rats when compared with the nicotine group ( $p < 0.05$ ). Malondialdehyde (MDA) level was increased and reduced glutathione (GSH) level was reduced in the nicotine group. The nicotine + DRLC group reduced the MDA levels and increased the GSH levels compared with the nicotine group. CRP and aspartate aminotransferase (AST) levels were significantly increased in the nicotine group. DRLC improved pregnancy outcomes by reducing blood pressure, oxidative stress and inflammation index altered by nicotine.

**Keywords:** D-Ribose-L-Cysteine, Fetal growth, Glutathione, Nicotine, Pregnancy

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### INTRODUCTION

Pregnancy is a delicate period in which the diet, lifestyle and other factors associated with the mother could have adverse effects on the development and growth of the fetus [1]. Chemicals and other environmental substances inhaled or ingested directly or indirectly contribute to the perturbation of the intrauterine environment [2]. Smoking cigarettes during pregnancy or exposure to second-hand smoke by the mother causes adverse pregnancy outcomes and it represents the first major environmental risk for the fetus [1, 3-5]. Significant risks such as ectopic pregnancy, placental previa and abruption, fetal growth restriction, preterm delivery, preterm premature rupture of membranes, oral facial clefts, and sudden infant death syndrome are some of the risk factors associated with smoking in pregnancy [6].

Tobacco smoke contains over 5,000 chemicals, including nicotine and 97 other hazardous components [7]. Nicotine, present in higher concentrations in tobacco and cigarette smoke has been implicated to be involved in smoking-induced toxicity [8]. It is a naturally occurring alkaloid in the nightshade family of plants [9], and it is currently used as a pharmaceutical drug to aid smoking cessation and relieve withdrawal symptoms [10]. However, because of its highly addictive nature, it becomes a potential problem for

pregnant women who smoke or are withdrawing from smoking [1]. The effects of nicotine in pregnancy are seen in every trimester of pregnancy, from increased spontaneous abortions in the first trimester, to increased premature delivery rates and decreased birth weights in the final trimester [11]. In animal models and humans, nicotine increases maternal blood pressure and heart rate, concomitantly reducing uterine blood flow. An increase in fetal heart rate is also seen, which is thought to be caused by catecholamine release [11].

There is an imbalance in the antioxidant system of pregnant women. This imbalance, favours oxidation and generation of free radicals which causes free radical damage in the mother and the growing fetus [12]. In an animal study, the administration of nicotine alone in pregnant rats heightened oxidative stress and vascular hypertensive reactivity [13]. Research has shown that maternal exposure to nicotine alone can lead to many negative consequences for the fetus [14]. Since there is an increase in the use of nicotine replacement therapy to stop smoking, and the addictive nature of nicotine means even pregnant women trying to stop smoking will find it difficult to do so. This study aimed at assessing the role of the novel intracellular glutathione producer; D-Ribose-L-Cysteine on the various toxicities induced by nicotine administration during pregnancy.

## MATERIALS AND METHODS

### Animal management

A total of twenty-eight female Sprague-Dawley rats were used for this study. Fourteen male rats were also used in the study for mating. The animals were housed in the college's animal facility, placed in well-ventilated

cages, and allowed access to feed and water ad-libitum. Proper hygiene and cleaning of their cages were done regularly. The animals were allowed to acclimatize for two weeks before the commencement of the study. The laboratory animals were taken care of and handled using the approved guidelines of the United States National Research Council [15].

### Animal grouping

The pregnant rats were randomly divided into four groups of seven rats each and administered nicotine and DRLC as described below;

Control	Received 10 mL/kg distilled water
Nicotine	Received 0.5 mg/kg body weight of nicotine intraperitoneally from day 4 of pregnancy to day 18.
Nicotine + DRLC	Received 0.5 mg/kg body weight of nicotine intraperitoneally and 200 mg/kg body weight of DRLC from day 4 of pregnancy to day 18.
DRLC	Received 200 mg/kg body weight of DRLC from day 4 of pregnancy to day 18.

### Assessment of oestrous cycle and mating of rats

The female rat's oestrous cycle was assessed for regular cyclicity over a period of 4 cycles. The wet method for the determination of oestrous cycle was employed [16]. Briefly, a dropper was used to drop small drops of normal saline in the rats' vaginas daily between the hours of 7 a.m. - 9 a.m. The dropper was used to collect the smear of the rats' vaginas and placed on a glass slide to determine the pattern of cells in the rat's smear under a light microscope. The pattern of the cells describes the various phases of the oestrous cycle the animal is undergoing. Animals in the proestrus phase were allowed to mate with male rats in separate cages. Smears were collected the following day from the rat's vaginas and the presence of sperm cells in the smear confirmed mating and was assumed as day 1 of pregnancy.

### Sacrificing of rats, blood pressure measurement and sample collection

On day 19 of pregnancy, the rats were anaesthetized with a solution of 25% (w/v) urethane and 1% (w/v)  $\alpha$ -chloralose intraperitoneally at a dose of 5 mL/kg. The trachea and the carotid artery were exposed and cannulated. Blood pressure measurements were obtained from the cannulated carotid artery connected to a physiological transducer. The transducer was connected to a power lab system (AD Instruments Pty Ltd., Castle Hill, Australia) and the systolic and diastolic blood pressure recordings was derived from the waveforms recorded from the power lab system. Recordings were taken at a sampling frequency of 5 Hertz.

Following the recording, blood was collected from the cannulated artery for the measurement of liver enzymes, bilirubin and C-Reactive protein levels. The abdominal cavity was also exposed and the numbers of the foetus, weights of the foetus and the placenta were

recorded. The placenta was also collected and stored in phosphate buffer for temporary storage before they were homogenized and used for oxidative enzyme studies.

### Determination of lipid peroxidation and antioxidant activities

The placentas were homogenized in phosphate buffer and used to assess lipid peroxidation and antioxidant enzyme activities. Malondialdehyde levels were measured using the TCA-TBA-HCl reagent (thiobarbituric acid (TBA) 0.37%, 0.24N HCl and 15% tricarboxylic acid (TCA) as previously described [17]. The activity of catalase was determined based on its ability to reduce  $H_2O_2$ . Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine and reduced glutathione in the placenta homogenate was also measured using established methods previously described [18-20]. All the absorbances were recorded using PG Instruments T70 UV/VIS spectrophotometer (Luthworth, UK) at the recommended absorbance wavelengths.

### Serum biochemical parameters measurements

Liver enzymes were measured in the serum of the rats using Randox kits. On day 17 of pregnancy, the animals were placed in metabolic cages that allowed water *ad libitum* and 12 hours of urine was collected and used to analyze total urine protein. The C-reactive protein level was determined using ELISA kits for CRP produced by ELABSCIENCE (China).

### Statistical analysis

The values reported in this study were analysed using one-way analysis of variance (analysis of variance) followed by students' Newman-keuls post-hoc test

using the graph pad prism (version 5.0). Differences were considered significant when  $p < 0.05$  and the values are expressed as mean  $\pm$  standard error of mean

## RESULTS

### Placenta and foetal weights

The pregnancy outcome of the rats administered nicotine and DRLC is shown in Table 1. The table shows no significant difference in the weight of the dams

across the groups. However, the placenta and foetal weights of the nicotine-administered rats and the nicotine + DRLC groups were significantly decreased when compared with the control. The nicotine + DRLC group restored placenta and foetal weights when compared with nicotine but not when compared with the control ( $p < 0.05$ ). Litter size was reduced in the nicotine group when compared with the control and DRLC co-administration with nicotine restored the litter size when compared with the nicotine group ( $p < 0.05$ ).

**Table 1:** Pregnancy outcome of rats administered D-ribose-L-cysteine and Nicotine

	Control	Nicotine	Nicotine + DRLC	DRLC
<b>Animal weight at sacrifice (g)</b>	191.5 $\pm$ 9.05	178.0 $\pm$ 3.59	196.66 $\pm$ 2.95	184.5 $\pm$ 8.56
<b>Placenta weight (Day 19) (g)</b>	0.61 $\pm$ 0.01	0.49 $\pm$ 0.01*	0.54 $\pm$ 0.01*#	0.58 $\pm$ 0.01#\$
<b>Foetal weight (Day 19) (g)</b>	4.55 $\pm$ 0.08	3.44 $\pm$ 0.11*	4.02 $\pm$ 0.08*#	4.77 $\pm$ 0.07#\$
<b>Litter Size</b>	8.00 $\pm$ 0.44	5.83 $\pm$ 0.30*	7.00 $\pm$ 0.44#	7.66 $\pm$ 0.33#

\*represents a significant difference from control, # represents a significant difference from Nicotine and \$ represents a significant difference from Nicotine + DRLC.

### Blood pressure of the pregnant rats

The blood pressure of the pregnant rats administered nicotine and DRLC is shown in Table 2. The table shows that the systolic and diastolic blood pressure of the rats administered nicotine were significantly increased when compared with the control ( $p < 0.05$ ).

Nicotine + DRLC significantly reduced systolic blood pressure when compared with the nicotine group. The mean arterial pressure was increased significantly in the nicotine-treated rats compared with the control. No difference was found in the heart rates of the pregnant rats across the groups.

**Table 2:** Blood pressure and heart rate of pregnant rats administered nicotine and DRLC

	CONTROL	NICOTINE	NICOTINE + DRLC	DRLC
<b>Systolic blood pressure (mmHg)</b>	116.84 $\pm$ 7.03	146.6 $\pm$ 7.38 *	125.87 $\pm$ 6.21 #	116.91 $\pm$ 6.47 #
<b>Diastolic Blood pressure (mmHg)</b>	88.89 $\pm$ 4.34	115.55 $\pm$ 5.2 *	100.5 $\pm$ 5.25	90.31 $\pm$ 6.62 #
<b>Pulse pressure (mmHg)</b>	27.94 $\pm$ 3.6	31.41 $\pm$ 3.74	25.37 $\pm$ 3.74	26.59 $\pm$ 1.88
<b>Mean Arterial Blood pressure (mmHg)</b>	98.11 $\pm$ 5.1	125.5 $\pm$ 5.3 *	108.26 $\pm$ 5.03	98.82 $\pm$ 6.1 #
<b>Heart rate (beats/min)</b>	406.11 $\pm$ 20.7	420.5 $\pm$ 22.26	430.28 $\pm$ 18.6	408.8 $\pm$ 21.7

\*represents significant difference from control, # represent significant difference from Nicotine

### Oxidative stress indices

Table 3 shows the MDA levels, as well as the SOD, GSH, and CAT activities in the placenta of rats administered nicotine and DRLC. The table shows that MDA level was significantly increased in the nicotine-administered rats and the nicotine + DRLC group when compared with the control. The result, however, showed that the nicotine + DRLC group showed a significant

reduction in MDA levels when compared with the nicotine-alone group ( $p < 0.05$ ). The reduced glutathione level was significantly reduced in the nicotine-administered group when compared with the control. The nicotine + DRLC group however showed a significant increase in reduced glutathione when compared with the nicotine group.

**Table 3:** Oxidative stress indices in the placenta of rats administered nicotine and DRLC

	CONTROL	NICOTINE	NICOTINE + DRLC	DRLC
<b>Malondialdehyde (<math>\mu\text{mol/ml}</math>)</b>	$0.16 \pm 0.01$	$0.34 \pm 0.02^*$	$0.22 \pm 0.01^{* \#}$	$0.16 \pm 0.02^{\#}$
<b>Superoxide Dismutase (units/mg protein)</b>	$6.11 \pm 0.65$	$4.14 \pm 0.22$	$5.75 \pm 0.67$	$6.07 \pm 0.74$
<b>Reduced glutathione (<math>\mu\text{mol/ml}</math>)</b>	$1.85 \pm 0.27$	$1.08 \pm 0.025^*$	$1.52 \pm 0.33^{\#}$	$1.97 \pm 0.032^{\#}$
<b>Catalase (units/mg protein)</b>	$42.6 \pm 5.63$	$46.16 \pm 3.6$	$45.39 \pm 5.2$	$44.64 \pm 4.7$

\*represents significant difference from control, # represent significant difference from Nicotine

### Liver enzymes, urine total protein, and C-reactive protein concentrations

Table 4 shows that there was a significant increase in the AST levels of pregnant rats administered nicotine when compared with the control ( $p < 0.05$ ). C-reactive protein

levels were also increased in the nicotine-administered rats when compared with the control. However, coadministration of nicotine and DRLC reduced the levels of CRP when compared with the nicotine group ( $p < 0.05$ ).

**Table 4:** Liver enzymes, bilirubin, urine total protein and C-reactive protein concentrations in the pregnant rats' serum and urine

	CONTROL	NICOTINE	NICOTINE + DRLC	DRLC
<b>ALP (iu/L)</b>	$145.56 \pm 10.4$	$185.68 \pm 23.1$	$152.61 \pm 20.6$	$132.2 \pm 7.8$
<b>AST (iu/L)</b>	$8.26 \pm 0.38$	$12.1 \pm 0.66^*$	$9.53 \pm 0.91$	$9.32 \pm 0.4^{\#}$
<b>ALT (iu/L)</b>	$9.6 \pm 0.33$	$12.33 \pm 1.03$	$11.5 \pm 0.93$	$10.26 \pm 0.73$
<b>Bilirubin (mg/dL)</b>	$0.53 \pm 0.03$	$0.51 \pm 0.04$	$0.53 \pm 0.03$	$0.60 \pm 0.02$
<b>Total Protein (g/L)</b>	$38.5 \pm 3.66$	$43.43 \pm 2.76$	$40.4 \pm 1.05$	$33.26 \pm 3.91$
<b>C-Reactive protein (ug/mL)</b>	$0.04 \pm 0.002$	$0.06 \pm 0.003^*$	$0.04 \pm 0.003^{\#}$	$0.038 \pm 0.004^{\#}$

\*represents a significant difference from control, and # represents a significant difference from Nicotine. ALP: alkaline phosphatase AST: aspartate aminotransferase; ALT: alanine transaminase

## DISCUSSION

Maternal smoking during pregnancy is associated with several adverse developmental outcomes in the offspring [21]. Nicotine a major component of cigarettes induces spontaneous abortion in the first trimester and is responsible for preterm births and small gestational-age babies in the last trimester [11]. Our study administered nicotine from day 4 of pregnancies in rats a period in which implantation of the fetuses occur. Reduced placenta weight, fetal weight and litter size were found in the nicotine-administered rats. This demonstrates and confirms previous reports that nicotine impacts pregnancy and affects the offspring's outcome. Nicotine crosses the placenta, and its concentrations have been found in the amniotic fluid and fetal blood of pregnant women who smoke or use nicotine replacement therapy [11, 14]. Low birth weight is a significant risk factor for the development of hypertension, diabetes and obesity later in life. Our study showed that when DRLC was administered alongside nicotine, placenta and fetal weight increased compared to the nicotine group, however, the values were still significantly lower than those of the control. This infers that DRLC played a significant role in mopping up the adverse effects of nicotine on the fetus during pregnancy and could thus protect the fetus from the adverse effects of nicotine exposure during pregnancy later in life.

Systolic and diastolic blood pressures were increased in the pregnant rats administered nicotine. In humans, smoking in the first trimester and cessation of smoking during pregnancy increased the risk of gestational hypertension significantly [22]. Fetal and neonatal exposure to nicotine has been reported to cause an increase in blood pressure later in the adult life of the offspring [23]. Findings from this study showed that DRLC administered in pregnancy significantly reduced the blood pressure increase by nicotine. Nicotine activates the sympathetic nervous system and releases catecholamines which cause acute increases in heart rate and blood pressure [24]. Nicotine also affects endothelial function which arises as a result of inflammation and oxidative stress impacts on the blood vessels [24, 25, 26]. The effect of DRLC on reduced blood pressure could be a result of its antioxidant effect thereby preventing oxidative stress and enhancing endothelial function.

In rats, nicotine has been reported to trigger oxidative stress in reproductive tissues such as the uterus, ovary and pituitary gland involved in hormone production [27, 28]. This study reports an increase in lipid peroxidation in the placenta and decreases in the antioxidant enzyme level of GSH in nicotine-administered rats. This portrays a state of oxidative stress and this result is in line with previous studies in

rats [28, 29]. Increased oxidative/nitrosative stress plays an important role in the onset of various vascular diseases [30]. DRLC, a compound that enhances the production of intracellular glutathione is a potent antioxidant that mops up free radicals from the cells. This could be the reason why blood pressure increased by nicotine was diminished by the administration of DRLC. Co-administration of nicotine and DRLC in this study reduced lipid peroxidation in the placenta tissues and increased the levels of GSH reduced by nicotine.

The liver plays a major role in the metabolism and detoxification of toxic substances from the body. Nicotine is primarily metabolized by the liver enzyme CYP2A6 to cotinine. It can also be metabolized by glucuronidation (primarily by UGT2B10) and N-oxidation (primarily by FMO3), and there may be small contributions of other enzymes [31]. Nicotine has not been associated with liver test abnormalities or with clinically apparent hepatotoxicity [32]. Results from this study showed no significant difference in ALP and ALT levels but AST levels were significantly increased in the nicotine group this implies that nicotine has the potential to cause liver damage. Another protein CRP synthesized by the liver in response to inflammation was also measured. Nicotine increased the levels of CRP while co-administration of DRLC with nicotine reduced the concentration of CRP in the liver. Studies have shown elevated levels of white blood cells in heavy smokers as well as CRP and another acute phase protein, fibrinogen [33, 34, 35]. In humans, patients with elevated basal levels of CRP are at an increased risk of hypertension, diabetes and cardiovascular diseases. Both inflammation and oxidative stress contribute negatively to high blood pressure and other cardiovascular diseases.

## CONCLUSION

Taking together, nicotine-induced increase in blood pressure of pregnant rats could be related to its effects as a pro-inflammatory and pro-oxidant agent. DRLC which reduced CRP levels an index for inflammation reduced oxidative stress indices as well as blood pressure could be said to have elicited its effect by its potential anti-inflammatory and antioxidant function. Thereby, improving pregnancy outcomes.

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